Claims

1. Method for determining absolute mRNA quantities by means of DNA microarrays with the following steps:
   a. providing a microarray;
   b. providing at least one or more dilution series of control spots on the microarray;
   c. hybridizing with a corresponding control DNA of known concentration;
   d. deriving reference data from the hybridization and
   e. using said reference data to calculate absolute mRNA concentrations in one or more samples used.

2. Method according to claim 1, further comprising the step of providing a universal tag at each of the immobilized probes on the array to be used for quantification.

3. Method according to claim 1 or 2, wherein a DNA microarray is a cDNA microarray and/or the control DNA is control cDNA.

4. Method according to any of the preceding claims, wherein the reference data comprise a model curve which is fitted or adapted to the obtained signals for control.

5. Method according to any one of the preceding claims, wherein cDNA templates for the genes that are to be probed for are first amplified by large-scale multiplex polymerase chain reaction (PCR) to obtain amplified fragments.

6. Method according to claim 5, wherein said amplified fragments are then transferred to the microarray by means of roboting devices which are able to deliver nanoliter quantities with a spatial precision of better than 100 µm.

7. Method according to any one of the preceding claims, wherein the reference data obtained from the hybridization, e.g., the read-out signal intensities from the dilution

series spots, are taken as a basis for calculating the parameters of a model function by means of non-linear least-squares fitting.

8. Method according to claim 7, wherein for the model function the following function is used:

$$\hat{I} = \frac{KI_0 e^{rc_p}}{K + I_0(e^{rc_p} - 1)}$$

where $\hat{I}$ refers to the modeled signal intensity, c_p refers to the probe (or DNA spot) concentration, K represents the asymptotic signal intensity for $c_p \to \infty$, I_0 is the asymptotic signal intensity for $c_p \to 0$, and r is a shape parameter.

9. Method according to any one of claims 4-8, wherein fitting of the reference data is done by gradient optimization procedures.

10. Method according to claim 9, wherein for non-linear fitting, the Newton-Raphson method is used.

11. Method according to any one of the preceding claims, wherein a critical probe function is used to determine the set of spots whose values need correction for the influence of spot DNA concentration, the critical probe concentration function being defined by

$$c_{crit} = \frac{1}{r}\left[\ln\frac{17(K - I_0)}{3I_0}\right].$$

12. Computer program product comprising program code means stored on a computer readable medium for performing the computable part of the method of at least one of the preceding claims when said program product is run on a computer.

13. System, particularly for performing the method of at least one of claims 1-11, comprising:
    a. a microarray containing at least one or more dilution series of control spots;
    b. means for hybridizing with a complementary control DNA of known concentration;
    c. means for deriving reference data from said hybridization and
    d. means for making use of said reference data for calculating absolute mRNA concentrations in one or more samples used.

14. System according to claim 13, further comprising means for providing a universal tag at each of the immobilized probes on the array to be used for quantification.

15. Use of a method according to at least one of claims 1-11, a computer program product according to claim 12 and/or a system according to at least one of claims 13 or 14 for determining absolute mRNA quantities on cDNA microarrays.

16. Method of absolute quantification of other biomolecules like DNA or proteins in a complex sample by means of methods according to any one of claims 1-11.